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Eco-Physiological Barriers and Technological Advances for Biodiesel Production from Microalgae

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1. Introduction

The combination of diminishing fossil fuel reserves (peak oil) and increasing prices of diesel provide a challenge to the majority of nations in terms of national energy security and ensuring sustainable energy supplies. Such pressing challenges have provided the impetus for an acceleration of renewable energy research to identify novel and innovative liquid biofuels for the future (IEA 2011). Any such liquid biofuels from renewable resources will need to have a lower environmental footprint than fossil fuel derived liquid biofuels in order to meet key sustainability criteria (Nuffield 2011). The most abundant available natural renewable resource on planet earth is solar energy. Photosynthetic organisms such as plants, algae (macro- and micro-algae) and some bacteria have been selected through evolution to convert solar energy to storable forms of energy. Such photosynthetic organisms can constitute a renewable resource which can effectively harvest and convert solar energy to a variety of energy-dense biofuels. In the case of microalgae, at least US\$ 300 million has been committed to facilitate phycology research on bioprospecting microalgal diversity and evaluation of the feasibility of different microalgal species and strains for biofuels production (Sheehan et al., 1998). The use of oil crops such as palm, soy, and oilseed rape as feedstocks for biodiesel production has provided the basis for the first generation of biofuels. However, the cultivation of plants on arable land for biofuel production can compete with the use of the same land for food and animal feed production – the so called “food vs fuel” land-use competition dilemma. In addition, biofuel crops also have significant water and nutrient requirements which can adversely affect their sustainability when Life Cycle Analysis (LCA) is conducting across their value chain. For instance, microalgal production systems reliant on dwindling freshwater supplies will face sustainability problems if they are scaled up (Wigmosta et al., 2011). As a result microalgal systems based on saltwater or waste water are likely to be more sustainable. One approach being pursued for circumventing the ‘food vs fuel’ dilemma associated with first generation

biofuel involves biological processing of lignocellulosic biomass as the basis for development of second generation biofuel systems. The development of commercial-scale efficient conversion technologies for exploitation of biological wastes as an obvious source for biofuel generation is a major focus of research and development efforts associated with this generation of biofuel. One of the third generation biofuels under development aims to exploit the photosynthetic capability of microalgae for the conversion of solar energy into energy-dense biomass. A major advantage of microalgae over the use of crop biomass for biodiesel production is the lower land area requirements for production of an equivalent amount of fuel. As understanding of microalgal genomes and biochemistry increases, opportunities are emerging for development of fourth generation biofuels where metabolic engineering of microbes leads to more effective domestication of microalgae for biofuel production. However, at present the commercial production of biofuels from microalgae is limited by a lack of effective systems for biomass production, harvesting, extraction, and recovery of oils that can economically integrate all operational units from growth through to biofuel product recovery. In this chapter, we discuss the limitations of individual operational units in the context of efforts underway to establish fourth generation microalgal biofuels that are economically and environmentally sustainable.

2. Microalgae biomass generation

2.1 Open cultivation

Large scale microalgal biomass production can be achieved either through open pond cultivation under natural sunlight or under the controlled conditions of a photobioreactor. In the USA, the history of mass production of microalgae dates back at least to 1953 with the production of *Scenedesmus* species in Washington. Many systems for cultivating microalgae on a large scale have been suggested in many countries including the USA, Germany, Japan, Israel, the UK, the Czech Republic and others. Typically, microalgae are first grown in inorganic nutrients and then, in a second phase, are cultivated is done using waste water streams.

Commercial cultivation of microalgae can be done in a range of different ways including (a) open cultivation using natural sunlight, (b) closed cultivation using natural light and (c) closed cultivation using artificial light (in photobioreactors). Each of these systems has advantages and disadvantages, and the choice of system depends on the degree of parameter control needed to produce the desired product and on the value of the end-product (Apt and Behrens, 1999). The most commonly used artificial open pond systems consist of large shallow ponds, tanks, circular ponds and raceway ponds (Ugwu et al., 2008). The construction and operation costs of such open cultivation systems are considerably less but are challenging to operate on a year round basis due to seasonal climatic variations. While open pond culture is cheaper than culture in closed photobioreactors (Borowitzka 1999), it is currently limited to a relatively small number of microalgal species. Rectangular ponds with a paddle wheel (raceway ponds) are the most widely used for the production of *Spirulina* sp., *Dunaliella salina* and *Haematococcus* sp. and currently represent the most efficient design for the large scale culture of most species of microalgae. Individual ponds are typically up to 1 ha in area, with an average depth of about 20- 30 cm (Andersen 2005). The need to provide adequate light to the algal cells and maintaining an adequate water depth for mixing of the microalgae are important considerations for determining the pond depth. The diurnal natural light cycle results in the exposure of microalgae to limiting,

saturating and over-saturating light conditions. High irradiances throughout the year and moderate temperatures are optimal for outdoor microalgae cultivation. For example, the geographical location of southern Spain with an average of 10-12 hours of sunlight per day, and a mean solar irradiance ranging from 400 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ during winter to 1800 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ is considered highly suitable for outdoor cultivation of microalgae. The maximal areal productivity of microalgae in outdoor conditions ranges from 20 to 30 $\text{gm}^{-2}\text{d}^{-1}$ (Cuaresma et al., 2011). To date, light-to-biomass conversion efficiencies of 1-4 % have been achieved for microalgae grown in conventional open pond cultivation systems. Because the scaling-up of microalgal biomass production in open raceway ponds is relatively easy, such systems are primarily considered for commercial applications. However, differences in weather variables such as solar irradiance, rainfall, and temperature significantly affect prospects for open cultivation of microalgae at different geographical locations. Temperature influences the rate of various reactions of photosynthesis (Raven, 1988). Therefore, microalgae exhibit an optimal growth within a narrow temperature range and die above a certain threshold temperature (Béchet et al., 2011). In addition, temperature is an important factor that affects the rate of evaporation from shallow algal ponds. In addition to changing the physical environment of open ponds, rainfall can lead to microbial contamination that inhibits microalgal growth (Hase et al., 2000).

The paddle wheels installed in open ponds are used to circulate the water, while compressed air can be introduced into the bottom of a pond to agitate the water, bringing microalgae from the lower levels upwards. Raceway channels are typically built in concrete or compacted earth, and are often lined with white plastic. During daylight, the microalgal culture is fed continuously in front of the paddlewheel where the flow begins. The biomass is harvested behind the paddlewheel, on completion of the circulation loop. The paddlewheel operates continuously to prevent sedimentation and flocculation (Chisti, 2007). The largest raceway-based biomass production facility currently occupies an area of 440,000 m^2 (Spolaore et al., 2006). This facility is owned by Earthrise Nutritional (www.earthrise.com) and is used to produce cyanobacterial biomass for food. In India, Pary Nutraceuticals (part of the Chennai-based Murugappa group) has been focusing on microalgal research and development and are commercially producing *Spirulina* for nutraceuticals.

2.2 Closed cultivation systems for microalgae

Several different closed systems using natural sunlight have been described for microalgae (Richmond et al. 1993, Molina Grima et al., 1995, Spektorova et al., 1997). In such systems, microalgae are grown in transparent glass or plastic vessels, and the vessels are placed under natural illumination. A higher surface to volume ratio is provided, so microalgal cell densities are often higher than in open ponds. However, these systems are also subject to variations in light intensity and temperature that make cultivation reproducibility problematic. In addition, removal of oxygen from the culture and the provision of adequate temperature control (especially if energy is required for cooling) pose a major problem with such closed systems. Large scale indoor cultivation using highly-controlled photobioreactors or fermentors have also been used successfully for microalgal biomass production. The wide range of different types of closed photobioreactors (PBRs) include vertical-column, flat-plate and tubular PBRs (Ugwu et al., 2008). These provide the ability to control and optimize culture parameters, and as a result such photobioreactors are suitable for culturing many different species of microalgae. The basic features which must be considered when designing a photobioreactor are: source of light, churning rate of algae (to avoid biomass

sedimentation and for uniform availability of nutrients and light), material for construction, CO₂ supply, and removal of O₂, pH and temperature control (Kaur et al., 2010). Whether, closed or open systems will be optimal for commercial cultivation of different species (or strains) of microalgae is difficult to determine. However, it is clear that photobioreactors will play a critical role to feed open ponds with a high-cell-density unialgal inoculum (Cheng et al., 2011).

3. Thermodynamic efficiency of photosynthesis in microalgae

Photosynthesis is a chemical reaction governed by the laws of thermodynamics. Assuming a microalgal cell as 'boundary' and the process of photosynthesis as 'system', then according to the law of thermodynamics, the two kinds of work associated with this chemical reaction are electrical work and work of expansion. In a biological system such as microalgae, the production of ATP derived by the transfer of charges across the biofluidic membranes can be called electrical work. The growth or increase in size of cell and cellular components (including oil bodies) is the 'work of expansion'. In the very familiar photosynthetic reaction (Albarrán-Zavala & Angulo-Brown, 2007);

$$\Delta G^0 = 2880.31 \text{ kJ/mol}_{\text{C}_6\text{H}_{12}\text{O}_6} \quad \text{at } \lambda = 680 \text{ nm}$$

3.1 Photosynthetic conversion efficiency

In outdoor cultivation systems, the microalgal biomass productivity derived through photosynthesis depends on the solar energy input. The estimated yearly average solar energy density, including both direct beam radiation and diffuse scattered radiation is 10,038 MJ/m²year. To account for non-sunny weather conditions, a more realistic theoretical maximum solar energy density is obtained after reducing this value by 10%, which corresponds to a value of 9034 MJ/m²year. However, the actual value will exhibit temporal and spatial variation depending on the geographical location and will generally be lower (Cooney et al., 2011). The fraction of the solar energy spectrum (S_{Earth} ~ 9034 MJ/m²year) is further reduced by 45% to calculate the value of photosynthetically active radiation (PAR) that supports photosynthesis (S_{EarthPAR} ~ 4065 MJ/m²year). PAR is expressed in terms of photon flux as it reaches surface of microalgal cells in the form of photons, the energy of which varies inversely with the wavelength. The upper theoretical limit for the average PAR spectrum photon flux energy (E_{MaxAvePAR}) is 0.2253 MJ/mol that corresponds to λ531 nm (green) (Weyer et al., 2009). Hence, the available photon flux reaching the earth surface and which is available for photosynthesis is calculated by the following formula (Cooney et al., 2011):

$$\text{PF}_{\text{PAR}} = \frac{S_{\text{EarthPAR}} \sim 4065 \text{ MJ / m}^2 \text{ / year}}{E_{\text{MaxAvePAR}} \sim 0.2253 \text{ MJ / mol photon}} = 18,043 \text{ moles photons / m}^2 \text{ year}$$

3.2 Maximum theoretical photosynthetic efficiency

The most cited values for maximum photosynthetic efficiencies in microalgae are in the range of 17-23% (Gordon & Polle 2007, Zemke et al., 2010). Cooney and coworkers (2011)

illustrate how to obtain the maximum theoretical value of photosynthetic efficiency from the available PF_{PAR} of 18,043 moles photons/ m^2 year. The theoretical maximum is calculated by considering both photon transmission (η_{PTE}) and photon conversion efficiencies (η_{PUE}) as 100%. Thus, according to the following equation:

$$\text{Photons utilized} = PF_{PAR} \times \eta_{PTE} \times \eta_{PUE} = 18,043 \text{ moles photons} / m^2 \text{ year} \times 1 \times 1$$

In a microalgal cell, these photons power the photosynthetic production of carbohydrates (CH_2O) which have an average energy content of 0.4825MJ/mole (Weyer et al., 2009). On average 10 photons are required to derive one mole of CH_2O . Hence, the total energy consumed during the photosynthetic conversion reaction is obtained as follows (Cooney et al 2011).

$$E_{CARB} = \frac{(18,043 \text{ moles photons} / m^2 \text{ year} \times 1 \times 1) \cdot (0.4825 \text{ MJ} / \text{mole})}{10} = 871 \text{ MJ} / m^2 \text{ year}$$

The estimated total photosynthetic efficiency during the conversion of PAR to microalgal biomass is calculated by dividing E_{CARB} (871 MJ/ m^2 year) and $S_{EarthPAR}$ (4065 MJ/ m^2 year), assuming that bioconversion of carbohydrates is 100% efficient. This gives a value of 21.4%, which is stated as the overall maximum theoretical photosynthetic efficiency relative to PAR. High lipid productivity depends on both the microalgal biomass areal productivity and the lipid content that can be generated from the microalgal strain. The lipid productivity is the most important factor influencing the cost of biodiesel production. High lipid content microalgal species and strains also favor the efficiency of biomass processing during oil extraction.

3.3 Technological innovations in illumination sources

3.3.1 Light Emitting Diodes (LEDs)

A light source with narrow spectral output that overlaps the photosynthetic absorption spectrum improves the energy conversion as the emission of light at unusable frequencies is eliminated. Light-emitting diodes (LEDs) are the only light source that currently meet this criterion. LEDs have the ability to produce high light levels with low radiant heat output and maintain useful light output for years. Thus, LEDs can have a very significantly longer life of 100,000 h as compared to 8000 h of fluorescent lights. These advantages make LEDs ideal for microalgal growth in controlled environments of growth chambers. The optimal wavelength conditions will vary from species to species of microalgae (Chen et al 2011). For example, the highest specific growth rate and biomass production from the photosynthetic cultivation of *Spirulina platensis* was obtained using red LED. The superimposed pattern of luminescence spectrum of blue LED (450-470 nm) and that of red LED (650-665 nm) corresponds to the light absorption spectrum of carotenoids and chlorophyll (Yeh & Chung, 2009). Therefore, the red LED favors microalgal growth but switching to illumination with blue LED improved the rate of astaxanthin production by *Haematococcus pluvialis* (Katsuda et al. 2004). Flashing light from blue LEDs is also a promising illumination method for *H. pluvialis* growth and astaxanthin production (Katsuda et al 2006). The use of flashing LED as sources of intermittent light in indoor algal culture can yield a major gain in energy economy comparing to fluorescent light sources (Matthijs et al., 1996). The research results by Nedbal et al. (1996) also suggest that algal growth rates in intermittent light can be higher than those in equivalent continuous light.

Red LEDs were found to reduce the average cell volume of *Chlorella vulgaris* without affecting the total biomass production (Lee and Palsson, 1994). However, under the exposure of fluorescent light, cells regained their normal size.

3.3.2 Optical fiber technology

The use of optical fibers as internal light sources could increase light efficiency whilst simultaneously reducing electricity consumption. For example, solar energy excited optical fiber requires only 1.0 kW-h of electricity (Chen et al., 2011). Spatial (i.e. orientation and dimensions of the photobioreactor) and temporal variations (i.e. due to weather conditions) greatly affect the availability of sunlight. Therefore, internal illumination by optical fiber is unstable. To circumvent this problem, Chen et al. (2011) have conceptualized a photobioreactor that combines optical fiber and multi-LED light sources with both solar panel and wind power generators. This has a potential to be developed into a commercially viable microalgae cultivation system with significantly reduced electricity consumption.

4. Limitations and improvements of photosynthetic biomass production of microalgae

4.1 Low light intensity and distribution

There are several key parameters which determine the microalgal productivity in a photobioreactor. These are lighting, mixing, water, CO₂ pressure, O₂ removal, nutrient supply, temperature, and pH (Kunjapur and Eldrige, 2010). Under nutrient-sufficient and optimal temperature conditions, the maximal culture productivity of photoautotrophic microorganisms is solely limited by the light (Richmond, 2004). The penetration of visible spectrum of light in the microalgal cultures decreases as the cell density increases (Figure 1). The appropriate intensity, duration, and wavelength of light must be provided to enhance the microalgal growth in photobioreactors. Supra-optimal light conditions lead to photoinhibition and sub-optimal light becomes a growth limiting factor. In both conditions, microalgal productivity will be lowered. The photosynthetic conversion efficiency of microalgae will generally be lower than theoretically expected under optimal conditions due to insufficient capacity to utilize the incident radiation (Zhu et al., 2008). The distribution of solar radiation over a greater photosynthetic area can spatially dilute the light in the light saturation zone, thereby reducing the mutual shading of the cells in the culture resulting in higher growth rate and lower accessory pigments content. The distribution of solar radiation can be increased by maintaining the surface to volume ratio as high as possible. The temporal and spectral distribution of irradiation and photon flux density is the main physical parameter that determines the photosynthetic productivity of microalgae. The solar conversion efficiency of microalgal mass culture grown under full sunlight is limited because of two reasons: 1) the photon absorption rate of the chlorophyll antennae of upper layers of cells far exceeds the rate of their utilization hence there is a loss of excess photons as fluorescence and heat leading to photoinhibition; 2) the deprivation of functional photons in the deeper cell layers, which is strongly attenuated due to the filtering effect of upper cells (Naus & Melis, 1991, Neidhardt et al., 1998). Gordon and Polle (2007) argued that a microalgal biomass productivity of 100 g m⁻² h⁻¹ could be obtained solely by improving the flux tolerance rather than by raising intrinsic photosynthetic efficiency.

Fig. 1. Effect of biomass concentration on the penetration of incident light into cultures of *Nannochloropsis* sp. (from Richmond 2004).

4.2 Improvements of intrinsic photosynthetic efficiency

The photosynthetic efficiency of microalgae can potentially reach its theoretical maximum, which is calculated to be about 9-10% of total incident solar energy or 20-22% of PAR, being converted into biomass (Beilen, 2010). Such projected ultrahigh microalgal biomass yields of 100 g dry weight m⁻²h⁻¹ can be realized in photobioreactors with sufficiently thin channels, ultradense cultures, and rapid light/dark cycles wherein optimal synchronization of photonic input with rate limiting dark reaction times is exploited (Gordon and Polle, 2007). However, this does not take into account the intrinsic conversion efficiency of photosynthesis, which is only likely to be improved upon through genetic engineering or synthetic biology. The integration of molecular and photobioreactor engineering is likely the only possible way of obtaining near-theoretical levels of algal biomass productivity while simultaneously augmenting lipid content. At the unicellular level, genetic modification of microalgal photophysiology could decrease light absorption, leading to enhanced availability of functional irradiance at the population level. The PSII and PSI in the light harnessing complex of green algae are associated with large numbers of chlorophyll a and chlorophyll b molecules, which are called antenna molecules. During photosynthetic biomass production in photobioreactors, high photon flux densities saturate the antenna molecules of upper cell layers with excessive photons which do not participate in the photosynthetic biomass production. These excess photons dissipate their energy as heat or fluorescence (photoinhibition) and reduce the overall solar to biomass conversion efficiency of the microalgal culture. Moreover, the lower layers do not receive appropriate amount of photons because of the

filtration of light by the cells of upper layer, which accounts for a further loss in the overall biomass productivities (Figure 2). Genetic modifications resulting in truncated chlorophyll antennae size could restrict the high photon absorption by the light harvesting complex. In this context, Polle et al. (2003) have cloned and functionally characterized the Chl antenna size regulatory *Tla1* gene in *Chlamydomonas reinhardtii*. The partially truncated chlorophyll antenna size of the *tla1* mutant prevents the over-absorption of irradiance by cells, thus avoiding wasteful heat losses (Polle et al., 2003). In *Dunaliella salina*, a highly truncated light-harvesting Chl antenna size resulted in aggravated photosynthetic productivity and greater oxygen production under mass microalgal culture (Melis et al., 1999). The *Stm3LR3* mutants of *C. reinhardtii* generated by RNAi technology demonstrated down-regulation of the entire LHC antenna system. The *Stm3LR3* mutant showed reduced fluorescence, increased photosynthetic quantum yield, increased resistance to photoinhibition and faster growth rate under high light levels (Mussgnug et al., 2007).

Fig. 2. A diagrammatic representation of wild-type and genetically truncated light harvesting complexes of microalgae. The incident light falling on the antenna molecules in the LHC are wasted as heat and fluorescence, while the lower layer cells are deprived of light. The modified TM has fewer antenna molecules in the TLHC that allows the absorption of light by the cells in the deeper layers. TM (thylakoid membrane), RC (reaction center), LHC (light harvesting complex), TLHC (truncated light harvesting complex).

5. Microalgal biomass harvesting

Conventional harvesting processes for microalgae include dewatering, extraction and purification of biomass. Bulk harvesting of microalgal biomass can be performed by centrifugation, flocculation, gravity sedimentation and/or filtration. Biomass harvesting is one of the most energy intensive processes, and can require high capital investments. Harvesting techniques tend to vary from species to species as various factors (namely density, size and the value of the microalgal end product) will typically inform the most

appropriate method. Acoustic focusing, hybrid capacitive deionization or electrophoresis and use of novel materials for conventional membranes and flocculent systems are amongst the range of new innovative strategies that are currently under investigation (Cheng & Ogden, 2011).

6. Biomass conversion to biodiesel

6.1 Lipid extraction and biodiesel formation from microalgal biomass

Conventional methodologies for lipid extraction involve the use of toxic organic solvents such as chloroform, methanol and hexane. While the solvent extraction process is effective it is difficult to adopt on a large scale. Novel methods for lipid extraction involve technologies such as acoustics, sonication, the use of mesoporous nanomaterials, and amphiphilic solvents (Cheng & Ogden 2011). Super critical fluid extraction has been reported to be safer and faster than the conventional solvent extractions (Andrich et al., 2005). Another technique of “milking” microalgae manipulates the hydrophobicity of the solvent system, which allows the extraction of lipids from living algal cells. A flat panel two-phase bioreactor designed by Hejazi & Wijffels (2004) was used in the milking process for *Dunaliella salina* production. In this process, microalgal cells grown under optimal growth conditions are stressed by excess light to stimulate the production of β -carotene, which is then extracted from the cells using lipophilic compounds. Important considerations for application of this “milking” process includes: a) cell wall and membrane properties of the microalgal strain; b) location and accumulation of the product inside the cell; and c) biocompatibility and chemical properties of the solvent used for the “milking” process (Hejazi & Wijffels 2004).

Lipid conversion to biodiesel can easily be achieved by chemical trans-esterification, enzymatic conversion, and catalytic cracking. Chemically, biodiesel is comprised of monoalkyl esters of fatty acids that are derived from triacylglycerols. These triacylglycerides can be produced from crop or microalgae oils, animal fats, and waste cooking oils. Such biodiesel is miscible with petroleum diesel and thus suitable blends of biodiesel-diesel can be obtained. These are denoted as BXX, where XX is the percent of biodiesel in the blend. For example, B40 is 40% biodiesel in a diesel-biodiesel blend (Tat et al., 2007).

6.2 Fuel properties of microalgal biodiesel

There are several properties which determine the suitability of biodiesel as a biofuel including cetane number, kinematic viscosity, cold flow and oxidative stability (Ramos et al, 2009). These properties are greatly influenced by the fatty acid compositions of the feedstock oils (Figure 3, 4). Therefore, to determine the best composition of biodiesel, it is necessary to study the lipid profile of potential biomass feedstocks. The most common fatty acid methyl esters present in most biodiesel are palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3) (Knothe, 2008). Biodiesel obtained upon trans-esterification of these common fatty acids has many advantages over petroleum-derived diesel fuel. However, there are several performance problems with biodiesel, notably poor cold flow properties, lower cetane number and insufficient oxidative stability (Knothe, 2009). Ignition delay time and combustion quality of a diesel fuel is determined by the cetane number. An adequate cetane number is required for better engine performance and a high cetane number is also associated with

biodiesel with good cold start properties and reduced NO_x exhaust emissions (Ramos et al., 2009; Knothe, 2009; Ladommatos et al., 1996).

Fig. 3. Fatty acid composition of various vegetable oil crop feedstocks. (Data from Ramos et al 2009). HOSME (high oleic sunflower methyl ester), PME (palm methyl ester), RME (rape methyl ester), SME (soy methyl ester), CME (corn methyl ester), OME (olive methyl ester).

Fig. 4. Fatty acid composition of different microalgal species (Huerlimann et al., 2010). Data for *Chlorella* sp and *Scenedesmus* sp obtained from the author’s investigations.

The cetane number of biodiesel can be determined using established standards such as ASTM D975 in the USA. Cetane is the common name for a long straight-chain hydrocarbon, hexadecane (C₁₆H₃₄), which is the high quality standard on the cetane scale with an assigned Cetane number of 100. In contrast, the low quality standard is a highly branched compound heptamethylnonane (C₁₆H₃₄) which exhibits poor ignition quality and has an assigned cetane number of 15 (Gopinath et al 2010). According to ASTM D975, conventional diesel fuel requires a minimum cetane number of 40 whereas a minimum of 47 has been prescribed for biodiesel (ASTM D6751) or (EN14214). For biodiesel, the cetane

number depends on the microalgal feedstock biomass from which the oil is derived and the alcohol that is used during the trans-esterification process. The cetane number decreases with increasing unsaturation and increases with increasing chain length without branching of the CH_2 moieties. However, the straight chain fatty acid methyl esters with carbon numbers of 6, 10, 12, 14, 16 and 18 show a non-linear increase with carbon number. Esters of saturated fatty acids (such as palmitic and stearic acids) give high cetane numbers. In summary, cetane number increases with chain length, decreases with unsaturation (or the number of double bonds) and decreases as double bonds and carbonyl group move toward the centre of the chain (Graboski & McCormik, 1998). Oils from different microalgal feedstocks will have different fatty acid compositions and such fatty acids are different with respect to the chain length, degree of unsaturation, or presence of other chemical moieties. While esters of long chain saturated fatty acid show higher cetane number, they can have a high cloud point that results in nozzle clogging (see below). On the other hand, esters of unsaturated fatty acid show low cetane number and are prone to oxidation. Among the non-algal biodiesel feedstocks, palm oil is reported to have the highest cetane number (61), whereas peanut, sunflower and corn oils typically have a cetane values of 53 (Ramos et al., 2009).

To improve the properties of biodiesel, it is necessary to enrich the biodiesel with fatty esters with desirable properties. Genetic engineering can provide important opportunities in this regard. For example, a soybean transgenic crop variety designated 335-13 has been genetically altered to increase concentration of oleic acid by more than 85%, with a corresponding reduction of palmitic acid content to less than 4% (Tat et al., 2007). Saturated and long chain fatty acids gave a high cetane number, which increases with increasing saturation and the chain length (Knothe et al., 2003; Bajpai & Tyagi, 2006; Knothe, 2009). Esters of highly unsaturated fatty acids such as linoleic (18:2) and linolenic (18:3) acids lower the cetane number (Knothe et al., 2003). According to Knothe et al. (2003) high cetane numbers were observed for esters of saturated fatty acids such as palmitic (C16:0) and stearic (C18:0) acids. Feedstock oils rich in these fatty acid compounds would have higher cetane values.

A higher content of polyunsaturated fatty esters in oil derived from the feedstock can reduce the quality of biodiesel upon storage due to oxidation in the presence of air, light, heat, peroxides, trace metals, or even the structural features of the fatty acids themselves. Thus, oxidation stability is another major issue affecting the use of biodiesel fuels (Ramos et al., 2009; Knothe, 2009). The number and position of double bonds in the chains of unsaturated fatty esters are both factors affecting the susceptible to autooxidation reaction (Bajpai & Tyagi, 2006; Frankel, 2005). Most biodiesel fuels contain significant amounts of alkyl esters of oleic, linoleic and linolenic acids, which influence the oxidation stability of the fuels. The relative rates of autooxidation for oleic acid methyl ester, linoleic acid methyl ester and linolenic acid methyl ester have been reported to be 1, 41 and 98 respectively (Knothe et al., 2005; Frankel, 2005; Ramos et al., 2009; Knothe, 2009).

The high viscosity of non-esterified vegetable oils leads to operational problems in diesel engines by increasing the level of engine deposits. Reduction of the high viscosity of vegetable oils is facilitated by the production of alkyl esters from the oil by transesterification. Since viscosity increases with decreasing temperature, handling of fuels in lower temperatures is facilitated by this lower viscosity as well (Knothe, 2009). This can be achieved by increasing the length and degree of saturation of the carbon chains (Knothe et al., 2005). Wax settling and plugging of filters and fuel lines are typical

problems associated with biodiesel fuels at low temperatures. The maximum temperature at which the first solids appear for a particular fuel is known as the cloud point (CP) and such solids can lead to fuel filter plugging. The pour point (PP) is typically a few degrees below the cloud point and represents the temperature at which the fuel can no longer be poured (Dunn & Bagby, 1996). Key properties of biodiesel fuels at low-temperature are determined by the cold filter plugging point (CFPP) and low-temperature flow test (LTFT) (Dunn & Bagby, 1996; Knothe, 2009). The cloud point (CP) and CFPP are included in the biodiesel standards but as “soft” specifications (Knothe, 2009). For instance, The cloud point in ATSM D6751 requires a report, while the CFPP in UNE-EN 14214 varies with time of year and geographic location.

The properties of biodiesel at low-temperature are correlated with the properties of individual fatty acids, which mostly depend on the saturated ester content. In contrast, the effect of unsaturated fatty acids is considered negligible (Imahara et al., 2006; Ramos et al., 2009). Saturated fatty acids have significantly higher melting points than unsaturated fatty acids and in a mixture saturated fatty acids will crystallize at higher temperature. Therefore, biodiesel fuels derived from fats or oils with significant amounts of saturated fatty compounds display higher values of CP and CFPP (Knothe, 2003).

7. Summary

Biofuels can broadly be classified as oxygenated (ethanol, biodiesel) and hydrocarbon biofuels (diesel, jet fuel and gasoline). Based on this classification, the different generations of biofuels are - 1st generation, where biofuels are obtained from natural vegetable oils and greases; 2nd generation of lignocellulosic biomass and algal derived fuels. Two biomass crops, *Jatropha* and *Camelina* bridged, the 1st and 2nd generations of biofuels. The next generations of biofuels will be based on the innovative technologies that improve the processing of biomass into various other types of biofuels and improving the existing feedstock species of biofuel using metabolic/ genetic engineering. For example, application of heat and pressure on algae/biomass/waste using innovative approaches like hydrothermal, catalytic and biological biomass conversions for the creation of cost-effective biofuels as a replacement for fossil fuels. Moreover, the next generation fuels are direct replacements for petroleum and are compatible with the existing infrastructure of the petrochemical industry. Genetic modification of microalgae improves their photosynthetic biomass conversion efficiency and hence can lead to higher biomass productivities, which is necessary for economic scalability. The improvements in the existing infrastructure for microalgae biomass production by photo engineering approaches will also play a key role towards the commercial application of next generation microalgae biofuels. In summary, the replacement of petroleum based fuels by bio-based products depends on several key factors, which include selection of the right bio-based product, process modification or product improvement for indirect substitutions, technological interventions to lower the cost of individual processing steps, scalability of biomass production and bioproduct delivery, and availability of sufficient and productive land.

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The book "Biodiesel: Feedstocks and Processing Technologies" is intended to provide a professional look on the recent achievements and emerging trends in biodiesel production. It includes 22 chapters, organized in two sections. The first book section: "Feedstocks for Biodiesel Production" covers issues associated with the utilization of cost effective non-edible raw materials and wastes, and the development of biomass feedstock with physical and chemical properties that facilitate its processing to biodiesel. These include Brassicaceae spp., cooking oils, animal fat wastes, oleaginous fungi, and algae. The second book section: "Biodiesel Production Methods" is devoted to the advanced techniques for biodiesel synthesis: supercritical transesterification, microwaves, radio frequency and ultrasound techniques, reactive distillation, and optimized transesterification processes making use of solid catalysts and immobilized enzymes. The adequate and up-to-date information provided in this book should be of interest for research scientist, students, and technologists, involved in biodiesel production.

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